

Rainfall frequency, not quantity, controls isopod effect on litter decomposition

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ARTICLE INFO

Keywords:

Carbon cycling
Detritivore
Macrofauna
Precipitation frequency
Precipitation regime
Rainfall pattern

ABSTRACT

Increasing climate variability is one of the dominant components of climate change, resulting particularly in altered rainfall patterns. Yet, the consequences of rainfall variability on biogeochemical processes that contribute to greenhouse gas emissions has received far less attention than have changes in long-term mean rainfall. In particular, it remains unclear how leaf litter decomposition responds to changes in rainfall frequency compared to changes in cumulative rainfall quantity, and if changes in rainfall patterns will differentially affect organisms in the decomposer food web (e.g., microbial decomposers that break down leaf litter through saprotrophic processes versus detritivores that directly ingest leaf litter). To address this knowledge gap, we disentangled the relative importance of cumulative rainfall quantity and rainfall frequency on both microbial- and detritivore-driven litter decomposition, using the isopod *Armadillidium vulgare* as a model macro-detritivore species and simulating rainfall in a full-factorial microcosm experiment. We found that microbially-driven decomposition was positively related to cumulative rainfall quantity, but tended to saturate with increasing cumulative rainfall quantity when rainfall events were large and infrequent. This saturation appeared to result from two mechanisms. First, at high level of cumulative rainfall quantity, large and infrequent rainfall events induce lower litter moisture compared to smaller but more frequent ones. Second, microbial activity saturated with increasing litter moisture, suggesting that water was no longer limiting. In contrast, isopod-driven decomposition was unaffected by cumulative rainfall quantity, but was strongly controlled by the rainfall frequency, with higher isopod-driven decomposition at low rainfall frequency. We found that isopod-driven decomposition responded positively to an increase in the weekly range of soil moisture and not to mean soil or litter moisture, suggesting that an alternation of dry and moist conditions enhances detritivore activity. Collectively, our results suggest that *A. vulgare* morphological and behavioral characteristics may reduce its sensitivity to varying moisture conditions relative to microbial decomposers. We conclude that the activity of microorganisms and isopods are controlled by distinct aspects of rainfall patterns. Consequently, altered rainfall patterns may change the relative contribution of microbial decomposers and detritivores to litter decomposition.

1. Introduction

Decomposition, the physical and chemical breakdown of organic matter, is one of the major fluxes of CO₂ from terrestrial ecosystems to the atmosphere (Schlesinger, 2005). Given the decomposition sensitivity to climatic conditions (Adair et al., 2008; Aerts, 1997; Swift et al., 1979), understanding the consequences of ongoing climate changes on decomposition is fundamental to predicting the feedback effect on atmospheric CO₂ concentrations. This critical challenge has spurred the development of studies investigating the sensitivity of litter and soil

organic matter decomposition to altered temperature (e.g., Davidson and Janssens, 2006; Fierer et al., 2005; Hobbie, 1996) and rainfall (e.g., Wieder et al., 2009; Yahdjian et al., 2006; Yuste et al., 2011). Yet, such studies typically focus on changes in the mean state of climate (e.g., increased average temperature; decreased cumulative rainfall), thereby omitting climate variability, one of the dominant components of climate change. Rainfall events in particular are expected to become more variable, with larger but less frequent rainfall events leading to enhanced probabilities of drought and heavy rains (IPCC, 2013). These predictions raise a critical question: for a given cumulative rainfall

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quantity, are large and infrequent rainfall events equivalent to smaller but more frequent ones at inducing and sustaining decomposition? Furthermore, it is unknown whether responses differ among groups of organisms in the decomposer food web (e.g., microbial decomposers that break down leaf litter through saprotrophic processes versus detritivores that directly ingest leaf litter).

The limited understanding of rainfall variability effects on decomposition derives in part from the difficulty of manipulating rainfall regimes in a manner sufficient to capture the complexity of current and future rainfall regimes. Partial rainout shelters consisting of elevated frames with gutters exclude a known proportion of each rainfall event, thus reducing cumulative rainfall but keeping rainfall frequency unchanged (e.g. [Shihan et al., 2017](#); [Yahdjian and Sala, 2002](#)). Alternatively, rainout shelters consisting in full roofs deployed during a subset of rainfall events reduce both cumulative rainfall quantity and rainfall frequency simultaneously, confounding the effect of each factor (e.g. [Taylor et al., 2004](#); [Vogel et al., 2013](#)). While such methods have improved our understanding of the consequences of reduced cumulative rainfall or seasonal drought on litter decomposition, they provide little insight on the rainfall variability effect on decomposition. To our knowledge, advances in understanding rainfall frequency effects on decomposition have been limited to work in arid ecosystems, where a few studies have applied artificial rainfall events by watering the soil with equivalent amounts of water delivered as either large and infrequent pulses, or small and frequent ones. One study by [Whitford et al. \(1986\)](#), reported that 25-mm cumulative precipitation in the Chihuahuan Desert accelerated litter decomposition when added as small weekly pulses, but not when added as larger monthly pulses. This suggests that smaller but more frequent pulses may sustain decomposition better than large infrequent ones. In contrast, in the Patagonian steppe, [Austin et al. \(2009\)](#) reported that litter decomposition was similar when litter received a 10-mm pulse once a week or 3.3-mm pulses three times a week. These conflicting results indicate that reduced frequency does not always lead to a decline in decomposition, but may depend on cumulative rainfall quantity. Recently, [Joly et al. \(2017a\)](#) reported that large, infrequent pulses induced lower litter decomposition than small, more frequent pulses, but only at high level of cumulative rainfall when the differences in pulse size were highest. This suggests that decomposition may saturate at a certain threshold in pulse size ([Fig. 1a](#)), leading to non-linear responses of decomposition to increasing cumulative rainfall quantity when delivered at low frequencies. Yet, the mechanisms underlying such saturation of decomposition remain unclear.

Another challenge in assessing decomposition responses to rainfall frequency is that studies to date have largely focused on microorganisms, omitting soil fauna responses. Studies have typically incubated litter over sieved and dried soil (e.g., [Austin et al., 2009](#); [Joly et al.,](#)

[2017a](#)) or within fine-mesh litterbags (e.g., [Whitford et al., 1986](#)). These methods may inadvertently affect research findings as soil fauna play an important role on litter decomposition, increasing mass loss by 37% on average across biomes ([García-Palacios et al., 2013](#)). Macrofauna are particularly important to litter decomposition, with detritivores such as millipedes catalyzing litter decomposition by fragmenting and compacting litter into fecal pellets ([Joly et al., 2018, 2015](#)). To date, the few studies assessing the response of detritivore feeding activity to altered rainfall pattern focused on drought intensity and duration, i.e. a change in both cumulative rainfall quantity and frequency. For instance, [Coulis et al. \(2013\)](#) found that millipede feeding activity decreased less than microbial activity in response to simulated drought (reduced watering amount and frequency). Similarly, in a four year field experiment simulating droughts with rainout shelters that reduced summer rainfall by 40%, [Thakur et al. \(2018\)](#) found that summer droughts did not reduce detritivore activity except when accompanied by increased temperatures. These results suggest that detritivore activity might be more resistant to dry conditions than the largely moisture-driven microbial activity ([Schimel et al., 1999](#)). Yet, in both studies the effect of frequency and cumulative rainfall were confounded. On the other hand, [Nielsen and Ball \(2015\)](#) hypothesized that greater soil moisture and hence large precipitation events are needed to trigger the activity of soil fauna relative to microorganisms. Such different responses are expected as small rainfall events that keep soil moist for short periods may be sufficient to trigger the activity of organisms with fast growth and reproduction time, such as microorganisms. However, larger rainfall events that keep the soil moist for longer time periods may be required to trigger the activity of larger organisms with slower growth and reproduction such as detritivores ([Schwinning and Sala, 2004](#)). In line with this reasoning, for a given cumulative rainfall quantity, detritivores should be less active with small and frequent events than with large, infrequent events, as a larger event would more likely trigger their activity ([Fig. 1b](#)). However, this hypothesis by [Nielsen and Ball \(2015\)](#) was put forward based on a synthesis of micro- and mesofauna studies, and was not tested experimentally. A better quantification of the sensitivity of distinct groups of organisms within the decomposer food web (e.g., microbial decomposers versus detritivores) to changes in rainfall frequency thus appears to be an important step toward improving predictive capability of the consequences of rainfall patterns on litter decomposition.

In this study, we aimed to determine the relative importance of cumulative rainfall quantity and frequency on microbial and detritivore-driven litter decomposition. In line with the aforementioned expected responses (see [Fig. 1](#)), we hypothesized that (H1) microbially-driven decomposition would increase linearly with cumulative rainfall, but would reach a plateau for the low rainfall frequency at high cumulative rainfall ([Fig. 1a](#)). Further, we hypothesized that (H2)

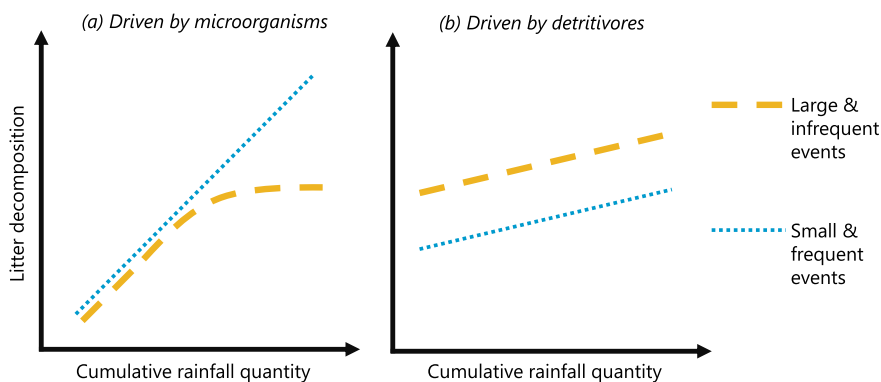


Fig. 1. Hypothesized relationships between cumulative rainfall quantity and decomposition driven by (a) microbial decomposers and (b) detritivores when rainfall is delivered as large and infrequent events (yellow dashed line) or small but frequent events (blue dotted line). (a) For microbial decomposer, we hypothesized that increasing cumulative rainfall quantity leads to a linear increase in microbial decomposition when delivered as small frequent events. When delivered as large but infrequent events, we hypothesized that microbially-driven decomposition saturates because litter moisture saturates and microbial activity is no longer limited by moisture. (b) For detritivores, we hypothesized an increase in detritivore-driven decomposition with increasing cumulative rainfall quantity, but with a shallower slope than for microbially-driven decomposition, as detritivores may be

more resistant to drought than microbes. We also hypothesized that large infrequent events lead to higher detritivore-driven decomposition than small and frequent events, as detritivores may require greater soil moisture to trigger activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

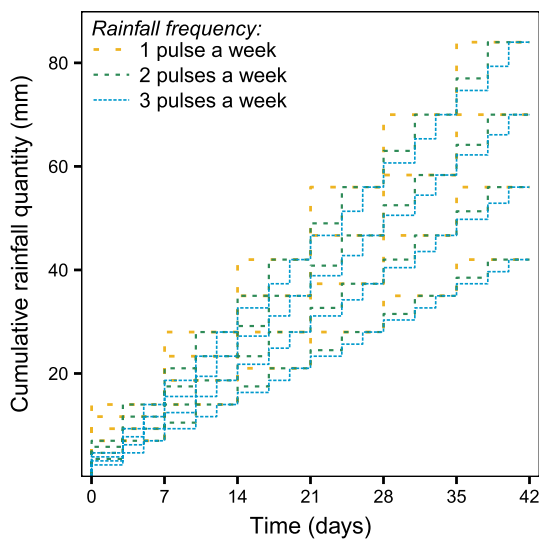


Fig. 2. Simulated rainfall patterns throughout the incubation for the different cumulative rainfall quantity (30, 40, 50 and 60 mm month⁻¹) delivered at three different rainfall frequencies (once a week, yellow dashed line; twice a week, green dashed line; three times a week, black dashed line). For a given cumulative rainfall quantity, less frequent pulses were also larger than the more frequent ones. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

detritivore-driven decomposition would increase with decreasing rainfall frequency and would be less sensitive to increasing cumulative rainfall than microbial decomposition (Fig. 1b). We tested these hypotheses by measuring litter decomposition of velvet mesquite (*Prosopis velutina*) in the absence or presence of detritivores, in addition to the presence of microbial decomposers, after a six-week incubation under controlled conditions. As an operational definition for this study, we defined decomposition as litter carbon (C) loss during the course of the experiment, regardless of whether from saprotrophic processes or ingestion by detritivores. Litter C loss in the absence of detritivores was used as an indicator of microbially-driven decomposition, while the increase in C loss with the addition of detritivores was considered as detritivore-driven decomposition. We used the isopod *Armadillidium vulgare* Latreille as a model detritivore species. Rainfall was delivered as a factorial combination of four cumulative rainfall levels and three rainfall frequencies (Fig. 2). To assess the effect of varying rainfall amounts and frequencies on soil and litter moisture and to assess the control of these variables over microbial and detritivore-driven decomposition, we monitored soil and litter water content throughout the incubation.

2. Methods

2.1. Detritivore, litter, and soil collection

We chose the common pill woodlouse (Isopoda: Armadillidiidae) *Armadillidium vulgare* (referred to as “isopods” hereafter) for our experiment, as it is a widespread detritivore species. This species is native to Europe and introduced in North America. It is widely distributed, existing in mesic temperate ecosystems as well as semi-arid ecosystems that undergo long periods of drought. We collected around 400 individuals in April 2017 from the litter layer of a tree-covered and drip-irrigated area landscaped with native Sonoran Desert species in Tempe, Arizona, USA (33°25'8" N, 111°55'39" W). The isopods were kept until the start of the experiment in containers containing soil and moist decomposing litter from the collection site.

We collected leaf litter of velvet mesquite (*Prosopis velutina* Woot.) in May 2017 from a Sonoran Desert mesquite shrubland (33°32'13" N,

111°25'56" W), near Phoenix, Arizona, USA. We used decomposing leaf litter rather than freshly senesced litter due to detritivore preference for partially decomposed litter (David and Gillon, 2002; de Oliveira et al., 2010). Collected leaf litter was air-dried, cleaned of debris (small rocks, twigs, non-targeted litter species, adhering soil particles), and stored in paper bags until the start of the experiment. Leaf litter C and nitrogen (N) concentrations were 0.392 g C g⁻¹ litter and 0.023 mg N g⁻¹ litter, C:N ratio was 17.0, and water-holding capacity was 1.64 g H₂O g⁻¹ litter.

Surface soil (top 5 cm) was collected in April 2017 at the Desert Botanical Garden (33°27'51"N, 111°56'26"W) in Phoenix, Arizona, USA, from inter-canopy areas of a mesquite shrubland. Soil was air-dried at 30 °C and passed successively through 5 mm and 2 mm sieves. We discarded the > 5 mm fraction and retained the 2–5 mm fraction (hereafter ‘fine gravel’) and the < 2 mm fraction (hereafter ‘soil’). The soil texture was loamy sand (71% sand, 25% silt, 4% clay) with a water-holding capacity of 0.339 g H₂O g⁻¹ soil.

2.2. Experimental setup

In a full-factorial experiment, we manipulated cumulative rainfall, rainfall frequency, and isopod presence. Cumulative rainfall treatments consisted of 30, 40, 50 and 60 mm month⁻¹ (for a total of 42, 56, 70 and 84 mm throughout the six-week incubation). The corresponding weekly amount for each cumulative rainfall treatment was delivered in three different frequency treatments: rainfall was added in one large pulse each week, two intermediate-sized pulses each week, or three small pulses each week (Fig. 2). Each cumulative rainfall and frequency treatment combination was applied to two decomposer treatments: one treatment with microorganisms only and one treatment with microorganisms plus four isopods. Each treatment combination was replicated 4 times for a total of 96 microcosms (4 cumulative rainfall levels x 3 frequencies x 2 decomposer treatments x 4 replicates).

All microcosms consisted of 450 ml plastic containers that were perforated on the bottom with 13 holes (1.5 mm diameter) to allow water drainage. Each microcosm was lined with an air- and water-permeable polypropylene cloth to prevent soil loss. A layer of 70 ± 1 g of fine gravel on top of the cloth facilitated drainage, and this was topped with 350 ± 1 g of soil. Because terrestrial isopods including *A. vulgare* commonly seek shelter during dry conditions to avoid desiccation (Dias et al., 2012), we constructed shelters (20 mm long and 5 mm high) made from plastic irrigation pipe cut lengthwise. We placed one shelter in each microcosm and then added 2.0 ± 0.01 g of air-dried leaf litter. Initial air-dried litter mass was converted into oven-dried mass by weighing air-dried litter subsamples, drying them at 60 °C for 48 h, and reweighing them to obtain dry mass. We ensured that no leaf litter was placed under the shelters to avoid preferential litter decomposition in the shelter microclimate. Four isopods were added to each isopod treatment microcosm, with individuals selected to have a total mass of 300 ± 50 mg without gender distinction. To prevent isopod escape while allowing evaporation from soil and litter surfaces, microcosms were covered with 2 × 2 mm nylon mesh secured with rubber bands. For all microcosms, the microbial community present was derived from microorganisms present in the air-dried soil and litter. Microcosms were incubated in a controlled environment chamber (20 °C, 50% relative humidity, 12 h day/night cycle) for six weeks. This duration was chosen as it allows substantial microbial decomposition even under dry conditions (Joly et al., 2017a) and to prevent litter from becoming limiting in the isopod treatment.

Another set of 48 microcosms without isopods (4 cumulative rainfall levels x 3 frequencies x 4 replicates) was set up to monitor soil and litter moisture three times a week throughout the incubation (hereafter ‘moisture control’). Litter water content was estimated by selecting three leaflets from the litter layer, weighing these immediately, drying at 60 °C for 48 h, and reweighing. This led to a sampling of ca. 54 leaflets per moisture control microcosm throughout the incubation. We

assumed that this decrease would have negligible impact on litter and soil water content as each microcosm was filled with ca. 200 leaflets. Soil water content was estimated by weighing microcosms, given the known initial soil dry mass for each microcosm. The water contained in the litter was considered negligible compared to the soil water content. From these estimates, we calculated the overall mean litter and soil moisture throughout the incubation, mean weekly minimum, mean weekly maximum, and mean weekly range of litter and soil moisture.

Water pulses were added to microcosms from all frequencies on Fridays, to twice and three times a week frequencies on Mondays, and to three times a week frequencies on Wednesdays (Fig. 2). Water additions took place after moisture content measurements. These pulses were applied to microcosms by adding the designated volume of deionized water minus 3 ml to the litter surface with a bottletop dispenser. The remaining 3 ml were added with a hand sprayer (volume controlled by weighing the microcosm), ensuring a consistent distribution of water on the litter layer for all treatments. All isopod treatment microcosms were checked for dead isopods three times a week. Occasional dead isopods were replaced with live isopods of known mass.

At the end of the experiment, isopods were weighed for final mass and released at their collection site. For each microcosm, the mean isopod mass throughout the incubation was estimated as the mean of initial and final measurements. For microcosms in which we replaced dead isopods with live ones, we estimated the mean isopod mass as the mean of initial, intermediate, and final measurements weighted by the duration between measurements. All microcosms except those from the moisture controls were dried in a glasshouse for 48 h at ca. 40 °C. The remaining leaf litter in each microcosm was collected, cleaned of foreign material (small rocks, soil particles, and feces), dried at 60 °C for 48 h, and reweighed. Decomposed litter from each microcosm and five samples of initial litter were pulverized with a ball mill (8000D, Spex CertiPrep, Metuchen, NJ, USA) and analyzed for C concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies, Inc., Valencia, CA, USA).

2.3. Response variables

Litter decomposition was expressed as the percentage of C lost from the litter during the incubation: $\%C \text{ loss} = (M_i \times C_i - M_f \times C_f) / (M_i \times C_i) \times 100$, where M_i and M_f are the initial and final 60 °C dry masses, respectively, and C_i and C_f are the initial and final litter C concentrations (% of litter dry mass), respectively. We used litter C loss rather than total litter mass loss to correct for inorganic contamination of leaflets retrieved from microcosms where they were in direct contact with soil. Since the initial litter consisted of decomposing rather than freshly fallen leaf litter, we assumed that most of the leaching, which

dominates the early decomposition stage (Berg and McClaugherty, 2014), had already occurred. We thus considered that C losses from the treatment without isopods resulted from microbially-driven decomposition. In turn, we considered that C losses from the treatment with isopods resulted from both microbially- and isopod-driven decomposition. To isolate isopod from microorganism effects on total C loss, we subtracted the C loss without isopods (mean value per treatment combination) to the C loss with isopods (Bocock's formula; David, 1998). This isopod effect was then corrected for differences in isopod mass among microcosms by dividing it by the isopod mass (average throughout the incubation) for each microcosm, providing a percentage of isopod-driven C loss per mass of isopod. We further multiplied this value by the mean isopod mass for all isopod microcosms (0.33 g), to ensure that isopod- and microbially driven decomposition were expressed with the same units, thus facilitating comparisons.

2.4. Statistical analyses

The hypothesized effects of cumulative rainfall quantity, rainfall frequency, and their interaction on (H1) microbially- and (H2) isopod-driven decomposition were tested using two-way ANOVAs. Additionally, the effects of cumulative rainfall quantity, rainfall frequency, and their interaction on soil and litter moisture were tested using two-way ANOVAs. For all ANOVAs, among-treatment differences were identified using Tukey HSD tests. We considered differences significant when $p < 0.05$ and differences marginally significant when $0.05 < p < 0.10$. Furthermore, we assumed that any effect of rainfall quantity and frequency over decomposition would be mediated by their effect on litter and soil moisture. Thus, to disentangle the underlying mechanisms, we used regressions to evaluate the control of litter and soil moisture (overall mean, mean weekly minimum, mean weekly maximum and mean weekly range) over both microbially- and isopod-driven decomposition. For each relationship, both linear and non-linear regressions (including asymptotic exponential, exponential, and power) were fitted and the best-fit model was chosen using the Akaike Information Criterion (AIC). All data were checked for homoscedasticity and normal distribution of residuals and transformed when needed. All analyses were performed using R version 3.4.4 (R Core Team, 2018).

3. Results

3.1. Microbially- and isopod-driven decomposition

Mean microbially-driven litter C loss after six weeks was $13 \pm 1.8\%$ (mean \pm SE) across all rainfall treatments. Litter C loss increased linearly with cumulative rainfall quantity when considered

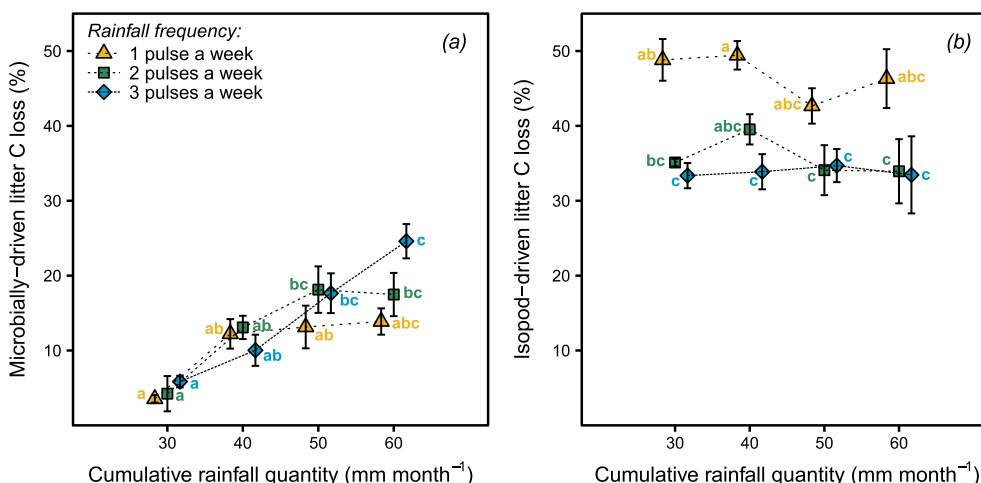


Fig. 3. Percentage of litter carbon (C) loss driven by (a) microorganisms and (b) isopods after six weeks of incubation (mean \pm SE, $n = 4$) under four different cumulative rainfall quantities (30, 40, 50 and 60 mm month⁻¹) and three different rainfall frequencies (1, 2 and 3 pulses per week). Microbially-driven litter C loss was the C loss in microcosms without isopods. Isopod-driven litter C loss was calculated for each microcosm as the difference in C loss between the litter loss with isopods and the mean C loss without isopods per treatment combination. Points on the cumulative rainfall quantity axis are jittered for clarity. Letters indicate significant differences in C loss among cumulative rainfall quantity and frequency treatment combinations (Tukey HSD tests).

Table 1

Results of two-way ANOVAs testing the main effects of cumulative rainfall quantity, rainfall frequency, and their interaction on (i) microbially-driven litter C loss ($n = 46$) and (ii) isopod-driven litter C loss ($n = 46$) after six weeks of incubation, and on (iii) litter moisture ($n = 48$) and (iv) soil moisture ($n = 48$). Soil and litter moisture variables were computed as the overall mean for all measurements taken per microcosm during the six-week incubation.

Source of variance	Microbially-driven litter carbon loss				Isopod-driven litter carbon loss			
	df	Mean sq.	F-value	p-value	df	Mean sq.	F-value	p-value
Cumulative rainfall quantity (CRQ)	3	471.7	24	< 0.001	3	26	0.8	0.478
Rainfall frequency (RF)	2	52.1	2.6	0.085	2	771.6	25.1	< 0.001
CRQ \times RF	6	33.2	1.7	0.155	6	17	0.6	0.765
Residuals	34	19.7	–	–	34	30.8	–	–

Source of variance	Litter moisture				Soil moisture			
	df	Mean sq.	F-value	p-value	df	Mean sq.	F-value	p-value
Cumulative rainfall quantity (CRQ)	3	15.7	79.6	< 0.001	3	395.2	153.867	< 0.001
Rainfall frequency (RF)	2	2	10	< 0.001	2	16.8	6.529	< 0.01
CRQ \times RF	6	0.9	4.5	< 0.01	6	0.5	0.183	0.979
Residuals	36	0.2	–	–	36	2.6	–	–

across all rainfall frequencies (Fig. 3a, Table 1). However, because rainfall frequency had a marginally significant effect ($p = 0.085$, Table 1), patterns of litter C loss were distinct for each frequency. While C loss linearly increased with cumulative rainfall quantity for the three pulses per week frequency, it plateaued with increasing cumulative rainfall quantity for the one and two pulses per week frequencies (Fig. 3a). Indeed, as indicated by the letters from the Tukey HSD test, when pulses were added once or twice a week, there were no significant differences in litter C loss between the 40, 50 and 60 mm month⁻¹ treatments (Fig. 3a).

Mean isopod-driven litter C loss was $39 \pm 1.8\%$ (mean \pm SE) across all rainfall treatments. In contrast to microbially-driven C loss, isopod-driven litter C loss was unaffected by cumulative rainfall quantity (Table 1), with no significant differences in C losses across cumulative rainfall quantity levels for a given frequency (Fig. 3b). However, isopod-driven litter C loss significantly decreased with increasing rainfall frequency (Table 1). This was particularly important at low levels of cumulative rainfall quantity (30 and 40 mm month⁻¹) for which C loss was significantly higher for the one pulse per week than for the three pulses per week treatment (Fig. 3b).

3.2. Changes in litter and soil moisture under altered rainfall pattern

Litter moisture increased with increasing cumulative rainfall quantity (Fig. 4a, Table 1). However, patterns of litter moisture differed among frequency treatments (Table 1) with litter moisture less

responsive to cumulative rainfall quantity for the one pulse per week frequency compared to the two and three pulse per week frequencies (Fig. 4a), as indicated by the significant interaction between cumulative rainfall quantity and frequency (Table 1).

Similar to litter moisture, mean soil moisture throughout the incubation significantly increased with increasing cumulative rainfall quantity (Fig. 4b, Table 1). In contrast to litter moisture, patterns of soil moisture were fairly similar among frequency treatments (Fig. 4b), but with significantly higher soil moisture at lower rainfall frequency (Table 1).

3.3. Moisture control over microbially- and isopod-driven decomposition

Microbially-driven litter C loss responded positively to litter moisture, with the best-fit model following an asymptotic exponential function (Fig. 5a). Soil moisture was also positively correlated with microbially-driven litter C loss, explaining 52% of the variance in C loss (Fig. 5c). All other litter and soil moisture variables (mean weekly minima, maxima and range) also positively correlated significantly with microbially-driven litter C loss (data not shown).

In contrast, isopod-driven litter C loss correlated neither with litter nor soil moisture (Fig. 5b, d). Of all litter and soil moisture variables (mean weekly minima, maxima, mean and range of moisture), the range of soil moisture was the only variable significantly related to isopod-driven litter C loss, explaining 11% of variation (Fig. 6).

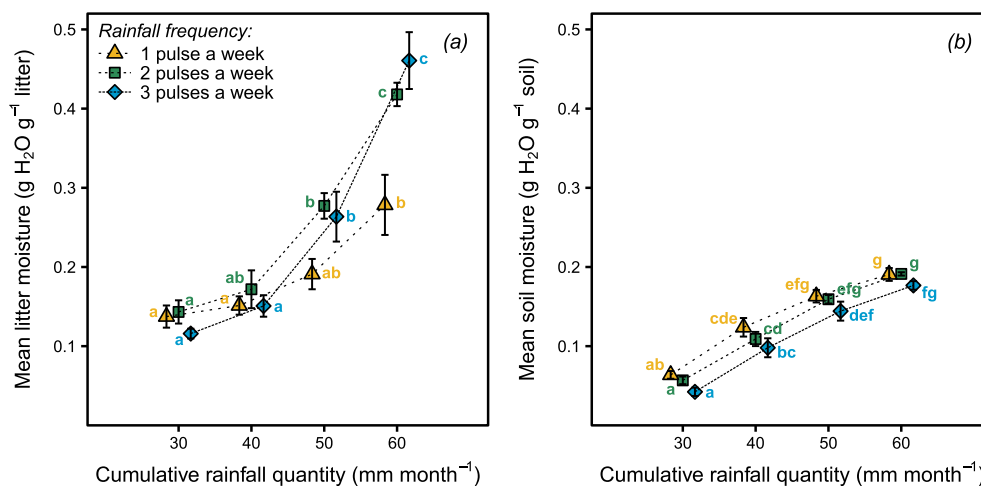


Fig. 4. Mean (a) litter and (b) soil moisture for the six-week incubation (mean \pm SE, $n = 4$), under four different rainfall quantities (30, 40, 50 and 60 mm month⁻¹) and three different rainfall frequencies (1, 2 and 3 pulses per week). Points on the cumulative rainfall quantity axis are jiggered for clarity. Letters indicate significant difference in C loss among cumulative rainfall quantity and frequency treatment combinations (Tukey HSD tests).

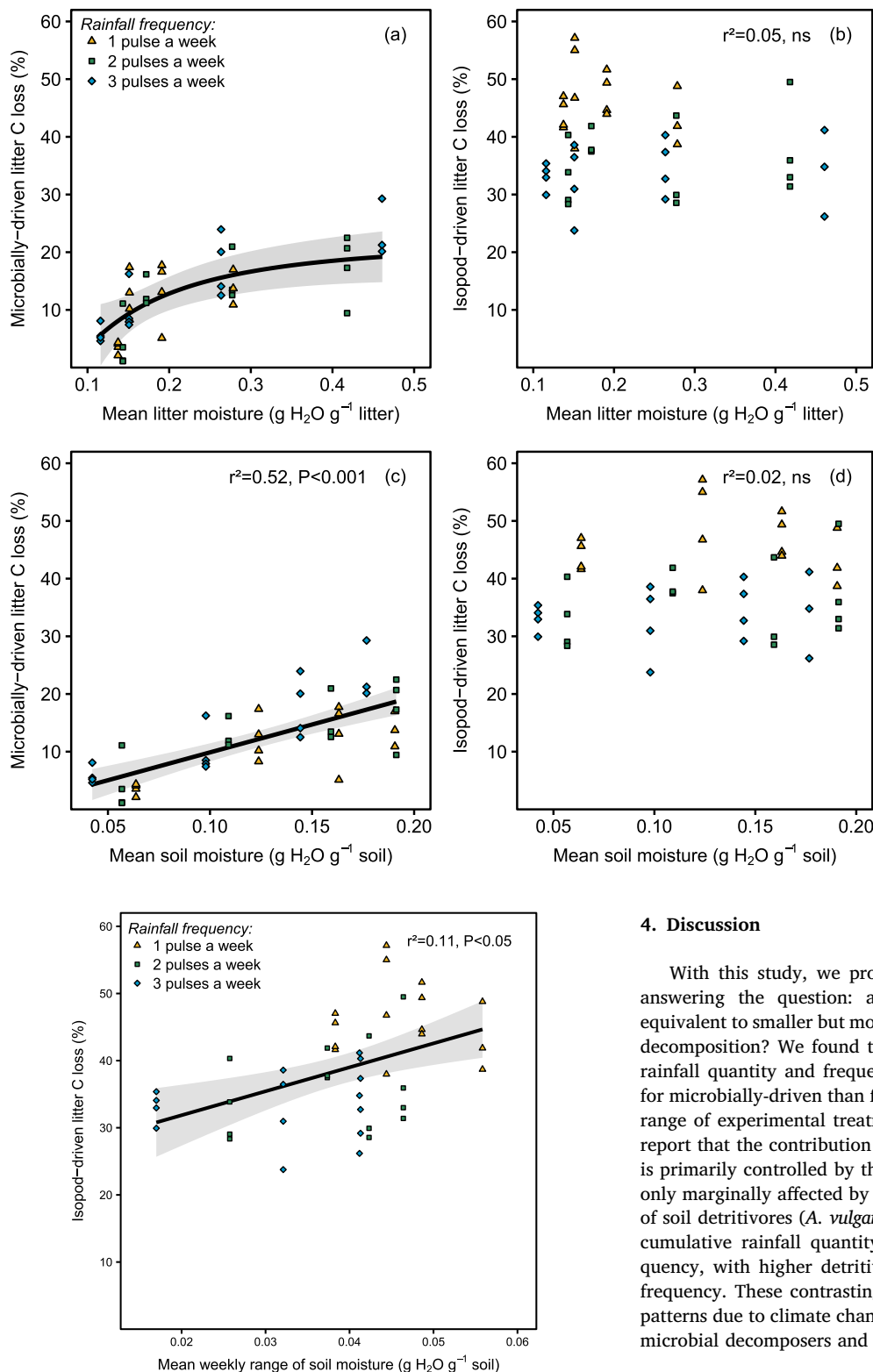


Fig. 6. Isopod-driven litter C loss as a function of the mean weekly range of soil moisture. This range of soil moisture was computed as the mean difference between the weekly maximum and weekly minimum soil moisture. The black line represents the linear regression line and the grey area represents the 95% confidence interval of the regression line.

Fig. 5. Microbially-driven litter C loss as a function of (a) mean litter moisture and (c) mean soil moisture, and isopod-driven litter C loss as a function of (b) mean litter moisture and (d) mean soil moisture. Mean moisture variables were computed from all measurements during the six-week incubation. A black line represents the regression line if the slope is significantly different from zero, and grey areas represent 95% confidence intervals of regression lines. The regression line on panel (a) correspond to an asymptotic exponential non-linear model, while the regression line on panel (c) correspond to a linear model. The r^2 and p -values are not reported for Fig. 5a as they cannot be computed for nonlinear regression. ns, nonsignificant.

4. Discussion

With this study, we provide some of the first elements to begin answering the question: are large and infrequent rainfall events equivalent to smaller but more frequent ones at inducing and sustaining decomposition? We found that the relative importance of cumulative rainfall quantity and frequency on litter decomposition was different for microbially-driven than for isopod-driven decomposition within the range of experimental treatments used for our study. Specifically, we report that the contribution of microorganisms to litter decomposition is primarily controlled by the overall cumulative rainfall quantity and only marginally affected by its frequency. In contrast, the contribution of soil detritivores (*A. vulgare*) to litter decomposition is unaffected by cumulative rainfall quantity, but strongly controlled by rainfall frequency, with higher detritivore-driven decomposition at low rainfall frequency. These contrasting responses suggest that changing rainfall patterns due to climate change may change the relative contribution of microbial decomposers and detritivores to litter decomposition.

4.1. Microbial response to changes in rainfall pattern

In line with our first hypothesis, microbially-driven decomposition generally increased with increasing cumulative rainfall. This control fits with the common view that macroclimatic variables, such as annual precipitation, are dominant drivers of litter decomposition (Meentemeyer, 1978). However, as expected based on recent findings (Joly et al., 2017a; Fig. 1a), microbially-driven decomposition increased linearly with increasing cumulative rainfall only when simulated rainfall events were delivered as small and frequent pulses

(Fig. 3a). In contrast, when delivered as large and infrequent pulses, microbially-driven decomposition appeared to saturate (Fig. 3a). The effect of rainfall frequency on microbially-driven decomposition was however only marginally significant (Table 1), contrasting with the higher effect of rainfall frequency observed in our previous experiment (Joly et al., 2017a). This may be due to the narrower range of cumulative rainfall quantity used in this study (30–60 mm month⁻¹), compared to the range (30–120 mm month⁻¹) used in Joly et al. (2017a), and to distinct environmental conditions such as lower temperature and higher relative humidity in the present study that may have reduced desiccation between rainfall events.

Saturation of microbial activity with increased cumulative rainfall quantity may result from three mechanisms. First, above a given threshold of event size, litter or soil may reach its maximum water-holding capacity with excess water lost to runoff or drainage. Second, increasing event size may not linearly increase the duration during which litter or soil is within the moisture range appropriate for microbial activity. Collectively, these mechanisms may limit the efficiency of rainfall to increase and sustain litter/soil moisture when rainfall is delivered as large and infrequent events. In line with this, we found that mean litter moisture was lower when pulses were large and infrequent, at high level of cumulative rainfall (Fig. 4a). In parallel, soil moisture tended to saturate with increasing cumulative rainfall quantity (Fig. 4b), which likely results from the fact that soil often reached its maximum water-holding capacity, and excess water was lost out of microcosms through drainage. As a third mechanism, microorganisms may no longer be limited by moisture conditions above a given threshold of litter and/or soil moisture. In line with this mechanism, we observed a saturation of microbially-driven decomposition with increasing litter moisture (Fig. 5a). Such saturation was previously reported for microbial respiration with increasing litter moisture (Schimel et al., 1999), with a decrease at very high levels of litter moisture. Such decrease was not observed in our study, possibly because we did not include a very high moisture treatment to prevent isopods from drowning. On the other hand, microbially-driven decomposition did not saturate with increasing soil moisture (Fig. 5c). However, because microbially-driven decomposition is likely to be more directly controlled by litter moisture rather than soil moisture, and because soil moisture also saturates with increasing cumulative rainfall quantity (Fig. 4b), it may be that the linearity of the relationship is coincidental rather than mechanistic. Nonetheless, soil microbial respiration may saturate with increasing soil moisture, as was reported by Sponseller (2007). Regardless of the underlying mechanisms, this saturation of microbial contribution to litter decomposition with decreasing frequency could have substantial consequences on C cycling and CO₂ emissions from terrestrial ecosystems.

4.2. Isopod responses to changes in rainfall pattern

In contrast to microbially-driven decomposition, isopod-driven decomposition was not affected by cumulative rainfall but was exclusively affected by rainfall frequency (Table 1). Isopod-driven decomposition was higher at low frequency (one pulse per week), than at high frequency (two or three pulses per week), particularly at low cumulative rainfall quantity (Fig. 3b). The lack of response of isopod-driven litter decomposition to cumulative rainfall quantity indicates that the activity of individuals from our studied population of *A. vulgare* was even more resistant to decreasing cumulative rainfall quantity than we had predicted (Fig. 1b) based on reports that soil fauna activity is relatively resistant to drought (Coulis et al., 2013; Thakur et al., 2018). This drought resistance may be due to strategies that limit desiccation, including morphological characteristics such as a lower surface:volume ratio compared to microorganisms, an exoskeleton, and, in the case of *A. vulgare*, the capacity to coil into a sphere (known as ‘volvation’). Additionally, mobility coupled with behavioral characteristics such as sheltering (Dias et al., 2012) may allow macrofauna to temporarily

relocate towards favorable conditions. Although relocation away from dry areas reduces the time spent on leaf litter foraging, it may allow satisfying water requirements by permitting water intake while minimizing water losses, as soil protected under shelters (e.g., rocks or wood in natural environments, artificial shelters in our study) may remain moist for longer than litter or exposed soil. Such spatial decoupling of food and water intake may allow isopods to remain active after the litter has dried down and as long as the soil in the shelter remains sufficiently moist. This behavior was reported for the desert isopod *Hemilepistus reaumuri*, which under dry conditions alternates feeding periods at the soil surface with resting periods in their moisture burrows (Shachak et al., 1979). In the burrows, *H. reaumuri* obtain water via cutaneous absorption of saturated air and by feeding on wet sand (Coenen-Staß, 1981). Our microcosms contained artificial shelters only, which may have offered some opportunity for mobility and behavioral responses to the moisture treatments. However, these responses may be even greater under field conditions than in our experiment, potentially leading to an even greater resistance to low moisture levels.

Greater isopod-driven decomposition at low rainfall frequency compared to high frequency fits our prediction that isopod-driven decomposition increases with decreasing rainfall frequency and associated increase in pulse size (Fig. 1b). This finding is in line with the theoretical framework put forward by Nielsen and Ball (2015) which suggests that a larger rainfall event that induces greater soil moisture is needed to trigger activity of invertebrates compared to microorganisms. However, we observed lower isopod-driven decomposition for the 60 mm month⁻¹/two pulses a week treatment than for the 30 mm month⁻¹/one pulse a week treatment (Fig. 3b), despite identical pulse sizes (7.5 mm per pulse) and lower average soil and litter gravimetric moisture content for the 30 mm month⁻¹/one pulse a week treatment (Fig. 4a and b). Furthermore, mean litter and soil moisture did not affect isopod-driven decomposition (Fig. 5b and d). Consequently, our results suggest that higher pulse sizes and average soil moisture alone are not sufficient to increase invertebrate activity – at least within the range of environmental conditions present for this experiment and for our studied species – in contrast with the hypothesis of Nielsen and Ball (2015). Instead, we found that the average weekly range of soil moisture (average difference between weekly maximum and minimum soil moisture) was the only moisture parameter related with isopod-driven decomposition (Fig. 6). This suggests that an alternation of dry and moist conditions is needed to maximize isopod activity. One possible explanation for this is that isopods such as *A. vulgare* perform compensatory feeding, consuming a higher quantity of moist leaf litter following periods of drought to satisfy their water requirements. Compensatory feeding has been reported for isopods feeding on low quality litter, i.e. consuming more low-quality than high-quality litter to compensate for its low nutritive value (Hättenschwiler and Bretscher, 2001), while moisture-related compensatory feeding has been reported for vertebrates (Nicolson and Fleming, 2003) but not isopods.

Collectively, our results based on the isopod *A. vulgare* suggest that rainfall frequency may have important consequences to detritivore-driven litter decomposition, in contrast to the important influence of cumulative rainfall for microbial litter decomposition. In ecosystems subject to long periods of drought that are interrupted with infrequent large rainfall events, slight changes in the size and frequency of these events may have important consequences on the activity of detritivores. In the Sonoran Desert, for instance, the litter consumption and survival of the desert millipede *Orthoporus ornatus*, which remains inactive for most of the year and emerges out of the soil only after monsoon rains (Wooten et al., 1975), may be jeopardized by a change towards more frequent but smaller rainfall events. On the other hand, a reduction in rainfall frequency and increase in event size in temperate ecosystems may lead to an increased contribution of soil macrofauna to litter decomposition.

4.3. Limits and ways forward

It is important to stress that we considered a single detritivore species in our experiment and our results may be specific to *A. vulgare*. The wide distribution of this species, spanning arid and mesic ecosystems, may explain its lack of sensitivity to cumulative rainfall quantity. Other detritivore species with narrower distributions, and/or with different physiological and behavioral reactions to desiccation may respond differently to changes in rainfall patterns. Indeed, desiccation resistance varies among detritivore species, including among isopod species, and was found to be higher in detritivore communities living in environments with low soil moisture than those in high soil moisture environments (De Smedt et al., 2018; Dias et al., 2013). Thus, detritivores less adapted to low soil moisture may still be affected by cumulative rainfall quantity, unlike *A. vulgare* in our study. Additionally, by comparing the response of a macro-detritivore to that of microorganisms, our study focused on the extreme ends of the decomposer food web size range. Intermediate-sized soil organisms (micro- and meso-fauna) may exhibit intermediate response to changes in rainfall patterns. In light of our results, assessing the response of other detritivores and other groups of the decomposer food web (e.g. microbivores, predators) to changes in rainfall patterns will be important for holistic prediction of litter decomposition. In addition, our experiment focused on the short-term responses of microbial and detritivore activities through their effect on litter decomposition. Over longer temporal scales, microbial and detritivore community composition may change as a response of rainfall pattern changes (David and Handa, 2010; Nielsen and Ball, 2015; Yuste et al., 2011). Notably, Zimmer (2005) reported that populations of four abundant isopod species in a floodplain forest in Germany were reduced by more intense precipitation during spring and dry summers. Understanding the consequences of rainfall variability on both community composition and feeding activity will thus be needed to predict the overall change in detritivore-driven decomposition. Given these limitations, we recognize that it is difficult to generalize our findings to field conditions where different detritivore species occur. Instead, we view our results as being most useful for testing hypotheses between rainfall pattern and the activity of different groups of the decomposer food web, and as a way to identify climatic controls over ecological processes that warrant further exploration *in situ*. Additionally, assessing the consequences of altered rainfall patterns in concert with changes in temperature is needed as these different aspects of climate change may have interactive effects on detritivore activity (Thakur et al., 2018).

5. Conclusions

Our study provides clear evidence that the contributions of different groups of the decomposer food web (i.e., microorganisms and a detritivore species) to litter decomposition are controlled by distinct aspects of rainfall patterns. While cumulative rainfall quantity was the dominant driver of microbially-driven decomposition, it did not affect isopod-driven decomposition within the range of treatments applied. In turn, rainfall frequency had contrasting effects on microorganisms and detritivores. At low rainfall frequency, microbially-driven decomposition saturated with increasing rainfall quantity, while isopod-driven decomposition peaked, particularly at low cumulative rainfall quantity. Change in rainfall frequency thus appear to be a largely overlooked aspect of climate change of importance to C and nutrient cycling of surface litter. In light of the recent reappraisal of the regulatory role of macroclimate over litter decomposition (Bradford et al., 2017, 2016; Joly et al., 2017b), our results highlight the need to consider climatic variables such as rainfall at smaller temporal scale and to consider the response of different groups of the decomposer food web that may have markedly distinct response to changes in rainfall patterns.

Acknowledgments

We are grateful to Daniel P. Fernandez and Rae M. Johnson for field and laboratory assistance and to Jean-François David for help with the identification of the isopod species. This project was funded by a grant from the National Science Foundation to HT (DEB 0953864) and an award to FXJ from the School of Earth and Space Exploration at Arizona State University's Great Pitching Contest. AW conducted this research as part of her undergraduate senior thesis.

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